VITAMIN E STATUS IN SWINE AS AFFECTED BY FORM OR LEVEL OF DIETARY VITAMIN E AND/OR BY SUPPLEMENTATION OF VITAMIN A

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1993

This dissertation is dedicated to my wife, Erma, and children, Valerie, Lee Jr., Jenaya, Calvin, and Kelvin, and to the memory of my parents, Florence Anderson, Myrtis Stepherson and Leon Anderson, for their love, support and encouragement.

ACKNOWLEDGMENTS

The author sincerely appreciates the efforts of all members of his supervisory committee. Dr. Robert O. Myer is greatly appreciated for his understanding, guidance, advice and companionship over the past several years. Sincere thanks go to Dr. Joel Brendemuhl for his help and guidance and his expertise and assistance during data collection. The author is very appreciative of Dr. Lee McDowell's help and advice, especially his assistance with acquiring the vitamins. The advice, assistance and encouragement of Dr. Jimmy Cheek and Dr. Joseph Conrad are deeply appreciated. Thanks go to Dr. George Combs for his assistance. Without the support of the above-mentioned individuals, this dissertation would not have been possible.

Special gratitude is kindly extended to Dr. Jack Fry, Dr. Roderick McDavis and Dr. Charles Kidd for their encouragement and assistance during the course of this program. Thanks are extended to Mr. Stephen Linda for the valuable assistance he has given in the statistical analysis of the data collected.

To his fellow graduate students, Maria Soler, Kim and Kelly Sheppard, Dave Davis and Ken Mooney, the author extends his thanks for their contributions and sacrifices in

assisting him in this research. Special appreciation is extended to Maria Soler and Kim Sheppard for their time and involvement in trial 3.

Deep appreciation is extended to Alvin Boning for his companionship and enthusiastic assistance in the laboratory. Thanks go to Nancy Wilkinson for her assistance in the laboratory. The author would like to take this opportunity to thank Dane Bernis, Tom Crawford, Dennis Perry, Jack Stokes, Dean Glicco and Rome Williams for their involvement in the preparation of diets, care and management of the experimental animals. Special appreciation is extended to Leroy Washington, Larry Eubanks and Art Rogers for their assistance in collecting tissue samples.

The author thanks Dr. Scot Williams and Hoffmann-La Roche for providing the vitamins used in trials 2 and 3.

A special thanks is also extended to Mary Chambliss for typing the manuscripts for review and publication.

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August, 1993

Chairman: R. O. Myer
Major Department: Animal Science

Experiment one used 40 finishing pigs (80 kg) to determine the potency of vitamin E compounds. Pigs were divided among five nutritionally adequate diets supplemented with DL-α-tocopherol, DL-α-tocopheryl acetate, D-α-tocopherol, D-α-tocopheryl acetate or no vitamin E. Blood and tissue samples were collected. Vitamin E forms increased ($P < .05$) serum α-tocopherol concentrations by d 2 of the feeding period. Serum tocopherol in pigs fed acetate forms remained elevated through out the study; serum concentrations declined ($P < .01$) in pigs fed alcohol forms. D-α-tocopheryl acetate resulted in highest serum and tissue tocopherol. The potency of D-acetate form was greater for swine than that predicted from bioassays with the rat.

Experiment two evaluated excessive dietary vitamin A on vitamin E status and performance of growing-finishing pigs.

Eighty-four pigs were fed corn-soybean meal based diets supplemented with DL-α-tocopheryl acetate to provide 0, 15 or 150 IU of vitamin E/kg and with retinyl acetate to provide 2,000 or 20,000 IU of vitamin A/kg of diet. Serum and tissue tocopherol increased ($P < .01$) as dietary levels of vitamin E increased. The data indicated that 20,000 IU of vitamin A/kg of feed did not affect performance or serum and tissue tocopherol.

In experiment three, 32 gilts were used to determine the effects of vitamins A and E on reproductive performance and on serum and tissue concentrations of vitamin E during early gestation. Treatments consisted of corn-soybean meal based diets supplemented with DL-α-tocopheryl acetate to provide either 25 or 500 IU of vitamin E/kg of diet, beginning d -7 prebreeding through d 25 of gestation. Half of the gilts were injected with 350,000 IU of vitamin A (retinyl palmitate) at d -7, again at breeding (d 0), and at d 7 postbreeding. Reproductive performance was not affected by treatment. Serum tocopherol increased ($P < .01$) with 500 IU of vitamin E. High (500 IU/kg) dietary vitamin E increased tocopherol level ($P < .01$) in all tissues except adipose. High vitamin A (350,000 IU) via injections had no consistent effect on reproductive performance or on serum or tissue concentrations of α-tocopherol or retinol.

CHAPTER 1
INTRODUCTION

Vitamin E was discovered in 1922 as a missing, needed dietary factor (Brandner, 1971; Ullrey, 1981; Raacke, 1983; McDowell, 1989). Vitamin E was isolated as alpha-tocopherol. The name tocopherol means to bring forth offspring (McDowell, 1989). George M. Calhoun, a professor of Greek at the University of California, Berkeley, named the new vitamin tocopherol in 1936 (tocos for childbirth, phero to confer, and ol for alcohol) (Evans, 1962; Ullrey, 1981; Raacke, 1983).

It was recognized in 1920 that reproductive failure occurred in rats consuming diets thought to be nutritionally adequate. An unknown dietary factor, then called X and later determined to be vitamin E, was deficient, which resulted in fetal death and embryo resorption in the laboratory rat (Evans, 1962; Mason, 1980; Diplock, 1985; McDowell, 1989). Estrus and mating were normal, but fetuses died and were resorbed unless the diet was supplemented with small amounts of wheat germ, dried alfalfa leaves, or fresh lettuce, which contained the deficient vitamin E (Evans, 1962; Mason, 1980; McDowell, 1989). Degeneration of the germinal epithelium in male rats was prevented by

supplements of fresh lettuce (Mason, 1980). Other animal species (cattle, sheep, mink, and chickens) were able to reproduce without dietary vitamin E, but in each case their offspring died prematurely (Brandner, 1971).

Vitamin E became known as the fertility vitamin. Many studies were done to determine if vitamin E affected reproduction in humans. In most cases vitamin E had little or no effect (McDowell, 1989).

Vitamin E deficiency in swine results in reduced reproductive efficiency, locomotor incoordination, muscular and hepatic necrosis, fibrinoid degeneration of blood vessel walls and muscular dystrophy (McDowell, 1977).

Vitamin E is a hydrophobic, peroxyl radical-trapping, chain-breaking antioxidant found in the lipid fraction of living organisms. Its principal function is to protect the lipid material of an organism from oxidation (Machlin, 1980; Burton et al., 1983; Diplock, 1985; McDowell, 1989; Coelho, 1991). Lipid peroxidation of membranes of cells and cellular constituents can be very damaging. Damage may be as simple as breaking a membrane and allowing leakage of contents, or as complex as breaking a membrane containing destructive enzyme systems. Hemolysis of red blood cells is an example of relatively simple membrane breakage. Membrane damage to lysosomes can be particularly devastating. Lysosomes are sometimes called the "suicide bags" of the cell, and when their membranes are broken they release

enzymes that hydrolyze tissue constituents and magnify tissue damage (Tappel, 1962). Damage to the membrane of such other cellular components as mitochondria and microsomes, which contain 25 and 40 % unsaturated lipid, respectively, have profound effects. In both microsomes and mitochondria, vitamin E is the only known lipid antioxidant (Tappel, 1962).

Selenium (Se) is a trace mineral that is known to spare some of the requirement for vitamin E. Selenium is a component of the enzyme glutathione peroxidase, which is a selenoprotein containing four atoms of selenium per molecule of protein (Scott, 1969; Draper, 1980). Glutathione is a hydrogen donor. Vitamin E functions as a fat soluble antioxidant, and selenium functions as a water soluble antioxidant (Cunha, 1977). Vitamin E is the first line of defense against peroxidation of fats in cells. If peroxides are formed, selenium through the enzyme glutathione peroxidase destroys the peroxides before tissue damage can occur. Thus, selenium is considered the second line of defense (Diplock, 1985; McDowell, 1989) and as a result, both selenium and vitamin E are capable of preventing some of the same nutritional diseases (McDowell, 1989). Vitamin E can also reduce the selenium requirement by inhibiting production of peroxides.

Pigs exhibiting clinical vitamin E and Se deficiency signs have a pale, white discoloration of the skeletal

muscle, an enlarged, friable heart, associated with hydropericardium, and sometimes intestinal edema and hepatosis dietetica (Mahan and Moxon, 1980).

There are many factors that affect the bioavailability of vitamin E. These include the form of vitamin E compound, potency of compound, stability, absorption, other fat soluble vitamins (e.g., retinol), mineral interactions, and unsaturated fat. Bioavailability is defined as the percentage of a drug or nutrient (in this case, vitamin E) that enters the systemic circulation after administration and the rate of entry into the general circulation for distribution throughout the body as well as tissue accumulation (Koch-Weser, 1974; "The American Heritage Dictionary", 1982).

Eight forms of vitamin E are known to occur in nature, four of which are referred to as tocopherols and four as tocotrienols. They have been given Greek letter names to distinguish them from one another (Diplock, 1985; NRC, 1988). The compounds differ in the placement of methyl groups on the ring and the degree of saturation in the side chain (McDowell, 1989).

Alpha-tocopherol is a yellow oil, soluble in certain organic solvents. It is common practice to assay only this isomer rather than all eight compounds because α-tocopherol is the most biologically active, naturally occurring vitamin E source (Ullrey, 1981).

DL-α-tocopherol has a potency of 1.1 IU/mg and its acetate (DL-α-tocopheryl acetate) has a potency of 1 IU/mg as determined by bioassays with rats. Activity of naturally occurring α-tocopherol, D-α-tocopherol (also called RRR-tocopherol) is 1.49 IU/mg and of its acetate, 1.36 IU/mg. D-α-tocopherol is the most biologically active form (IU per unit of weight; NRC, 1988).

Loss of vitamin E potency occurs in mixed feed from a number of factors. The naturally occurring tocopherols have relatively poor stability during processing, grinding, pelleting, and storing at high temperatures or under moist conditions. Vitamin E will also readily interact with other ingredients in feed formulations (Adams, 1978; NRC, 1988).

More pigs are being raised in confinement without access to pasture, which is an excellent source of vitamin E. Heating and pelleting feed grains lower their vitamin E values. The use of high moisture grain increases the need for vitamin E supplementation due to the destruction of the vitamin. Feeds formulated with fats containing high quantities of unsaturated fatty acids are susceptible to rancidity, which destroys vitamin E (Cunha, 1977). Malm et al. (1976) reported that diets high in polyunsaturated fatty acids increased vitamin E requirement and that pigs fed a polyunsaturated fatty acid, low vitamin E diet throughout the postweaning period resulted in some degree of red blood cell destruction.

The alcohol form, α-tocopherol, is easily destroyed by oxidation. Oxidative destruction of α-tocopherol is accelerated by heat, light, moisture, unsaturated fats, sulfates, nitrates and molds, and in diets containing increased levels of copper, iron, zinc and manganese (Ullrey, 1981; McDowell, 1989; Dove and Ewan, 1991; Mahan, 1991; Thompson, 1993). A more stable source of vitamin E is α-tocopheryl acetate. Alpha-tocopheryl acetate is chemically synthesized by esterification of α-tocopherol with acetic acid. DL-α-tocopheryl acetate is the international standard for vitamin E activity.

Vitamin E is fat soluble and as such its absorption is associated with that of lipids. Vitamin E is absorbed in the alcohol form. Vitamin E acetate is hydrolyzed to the alcohol form in the small intestine prior to absorption. Droplets of triglycerides are degraded by lipase and bile into monoglycerides and free fatty acids, which form into micelles. Micelles contain the lipid components including the fat soluble vitamins. Vitamins are absorbed with the fatty acids and monoglycerides. Triglycerides are re-formed in the intestinal cell and packaged into chylomicrons. Chylomicrons are absorbed into the lacteal ducts and carried into the lymphatic system until they enter the general circulation and are distributed to various tissues. Factors interfering with digestion and absorption of lipid affect the bioavailability of vitamin E.

Competition for absorption sites in the small intestines among the fat soluble vitamins may affect bioavailability of vitamin E. Vitamin A (retinol) may interfere with both absorption and blood concentrations of vitamin E. This has been demonstrated in chicks (Sklan and Donoghue, 1982; Abawi and Sullivan, 1989) and rats (Blakely et al., 1991). This effect appears to be due to increased oxidation of vitamin E prior to the digesta reaching the duodenum. This would result in vitamin E concentration being lower at the major absorption sites in the upper small intestine (Sklan and Donoghue, 1982). In this case, vitamin E is oxidized at the expense of vitamin A. Erdman et al. (1988) reported that vitamin E may protect vitamin A from oxidation in the gastrointestinal tract and within cell membranes. Reports that vitamin A toxicity in chicks has been completely reversed with high dietary vitamin E supplementation (Arnrich and Arthur, 1980) also indicate an additional loss of vitamin E resulting in an increased need. Young children, who were vitamin A deficient, absorbed more vitamin A when given high supplemental levels of vitamin E (Kusin et al., 1974) indicating that vitamin A may also affect the availability of vitamin E. However, there is very little or no research regarding the influence of vitamin A on the vitamin E status of swine.

Recently there has been an interest in increasing supplemental vitamin A levels via i.m. injections in

gestating gilts and sows. Extra vitamin A given just before, during, and shortly after breeding has been reported to improve reproductive performance in breeding swine (Brief and Chew, 1985; Coffey and Britt, 1993). The elevation of maternal plasma vitamin A is believed to improve embryonic survival (NRC, 1988). The elevated vitamin A may also affect bioavailability of vitamin E and/or its requirement.

Selection for increased growth rate and reproductive performance increases dietary vitamin E requirements. In addition, confinement rearing and feeding cereal-soybean meal diets that vary considerably in vitamin E content, make it important to insure that adequate levels of nutrients are included in the diet. Fortification of diets adequately supplemented with vitamins is extremely important in optimizing performance under current production conditions. More research is necessary to ascertain the significant aspects of vitamin E and its enhancement or impediment on performance under conventional swine production systems. Therefore the focus of this manuscript is on the bioavailability of vitamin E as affected by the type of vitamin E compound fed, and the influence of vitamin A supplementation on vitamin E status of growing-finishing pigs and during early gestation of gilts.

CHAPTER 2
POTENCY OF VARIOUS VITAMIN E COMPOUNDS FOR FINISHING SWINE

Introduction

Vitamin E is an essential nutrient for normal growth, health and reproduction in swine. Vitamin E requirement for swine ranges between 10 and 22 IU/kg of diet (NRC, 1988). Swine diets consisting mainly of corn and soybean meal usually do not contain adequate amounts of vitamin E needed to meet the pig's requirement (NRC, 1988). In addition, the stability of all naturally occurring vitamin E forms are very poor in mixed feed (Ullrey, 1981; Dove and Ewan, 1991; Hidiroglou et al., 1992). Therefore, supplementation of swine diets with a readily available form of vitamin E assures that swine will receive the correct amount for optimum performance.

Eight forms of vitamin E occur in nature (4 tocopherols, 4 tocotrienols). D-α-tocopherol has the greatest biological activity (highest IU per unit weight; NRC, 1988) but acetate and succinate forms are more stable (Erdman et al., 1988). This experiment evaluated the relative biopotencies of four forms of vitamin E (DL-α-tocopheryl acetate, D-α-tocopheryl acetate, DL-α-tocopherol, and D-α-tocopherol) when supplemented in the diet of

finishing swine. The concentration of α-tocopherol in blood serum and tissue was used as an indicator of potency.

Experimental Procedures

Forty crossbred finishing pigs, 20 barrows and 20 gilts, with an average initial weight of 80 kg were randomly assigned by sex to individual pens. Treatments were randomly assigned to the pens such that each treatment consisted of 8 pigs (4 barrows, 4 gilts). Treatments consisted of the following supplemented vitamin E forms: DL-α-tocopherol, DL-α-tocopheryl acetate, D-α-tocopherol, and D-α-tocopheryl acetate. A negative control, which received no supplemental vitamin E, was also included to give a fifth treatment. Vitamin E forms used were pure forms supplied in an unprotected oil solution (Sigma Chemical Co., St. Louis, MO). Supplemental vitamin E was added to the diets such that pigs consuming 3.2 kg of feed would received 200 IU per day. Pigs were fed a corn-soybean meal finishing diet formulated with a modified vitamin premix (exclusive of vitamin E) and .1 ppm of added selenium. Diets were otherwise formulated following NRC (1988) guidelines. Composition of the corn-soybean meal basal diet is given in Table 2-1. The pigs were fed the finisher diet for 28 days. Prior to the start of this trial all pigs were fed a diet that contained 22 IU of vitamin E/kg of diet. Feed and water were available ad libitum throughout the experiment.

Table 2-1. Composition of finisher diet

Ingredient	%, as fed
Ground corn	82.05
Soybean meal (48%)	15.00
Dynafos	1.70
Ground limestone	0.80
Salt	0.25
Trace mineral[a]	0.10
Vitamin mix[b]	0.05
Se premix[c]	0.05

[a]Provided 200 ppm zinc, 100 ppm iron, 55 ppm manganese, 11 ppm copper, and 1.5 ppm iodine.

[b]Provided 2.2 mg riboflavin, 11 mg niacin, 9 mg pantothenic acid, 150 mg choline chloride, 11 ug vitamin B_{12}, 1.5 mg vitamin K, 2750 IU vitamin A, and 440 IU vitamin D_3 per kg of diet.

[c]Provided 0.1 ppm selenium.

Pigs were housed in an open-sided building with solid concrete floors. Individual pig weights and feed consumption were recorded biweekly. The trial was carried out in the spring (April-May) of the year. Pigs were managed according to acceptable management practices throughout the experiment. Protocol for animal care had been approved by the University Animal Use Committee.

Blood samples were collected by jugular vein puncture from each pig on d 0, 1, 2, 7, 14, 21, and 28 of the feeding period. Blood samples were centrifuged after collection, and serum was frozen and stored at -20°C until analyzed. Feed samples were taken from the feeders on d 0, 5, 14 and 21, frozen and stored at -20°C until analyzed for α-tocopherol concentration. On d 29, the 20 barrows were slaughtered, using accepted slaughter procedures, at the University of Florida meats laboratory and tissue samples collected. Tissue samples included liver, muscle (rhomboideus and semimembranosus), back fat (10th rib area) and leaf fat. Tissue samples were frozen following collection and stored at -20°C until analyzed.

Procedures used for the extraction and determination of α-tocopherol in blood serum were as previously described (Njeru et al., 1992). Procedures were similar to those used by McMurray and Blanchflower (1979a,b) except in our study, propanol was used in the serum extraction instead of ethanol. Most of the vitamin E activity in serum and tissue

was assumed to be α-tocopherol (Ullrey, 1981). Extraction of vitamin E from tissues and feed was done using a procedure outlined by Chung et al. (1992). This procedure was a modification of that of McMurray and Blanchflower (1979b) and Hatam and Kayden (1979).

Alpha-tocopherol concentration was determined using 50 ul of the reconstituted sample (serum, tissue or feed) injected onto a LiChrosorb SI 60 column (Hibar Fertigsaule RT pre-packed column RT 250-4 E, Merck, Darmstadt, Germany) 250 mm x 4 mm I.D. and using a Perkin Elmer 550 terminal (Perkin-Elmer Corp. Analytical Instruments, Norwalk, CT), a Perkin Elmer ISS-100 auto sampler, and a Perkin Elmer Series 4 Liquid chromatograph pump. The mobile phase consisted of 900:99:1 HPLC grade iso-octane, tetrahydrofuran and acetic acid. The detector was a Perkin Elmer LS-4 Fluorescence Spectrometer with an excitation wavelength of 290 nm and an emission wavelength of 320 nm. Data were collected by a Perkin Elmer LCI-100 Laboratory Computing Integrator. Flow rate was 1 ml/min. The retention time of α-tocopherol was 5.2 minutes. Alpha-tocopherol (Eastman Kodak Company, Rochester, NY) was used as a standard, and sample peaks and retention times were compared to those of the standards. Standard concentration was calculated to give a peak of 250 or 500 ng. Alpha-tocopherol concentration of samples was calculated by the external standard method. Spiked samples were found to have a mean recovery rate of 97%.

Potencies of the various vitamin E compounds were determined by comparing areas under the time curve (AUC) within the serum, feed and tissue samples. Serum and tissue concentrations were adjusted based on actual feed intake and α-tocopherol levels in the feed. Alpha-tocopherol concentrations reported were adjusted to a constant feed α-tocopherol concentration (d 0 feed level). Serum and tissue means were analyzed using the general linear model procedure (SAS, 1988). Analysis of variance compared treatment differences in serum and tissue tocopherol concentrations. Analysis of covariance was also applied to the serum data using baseline (d 0 serum tocopherol) data as a covariate. Treatment means were compared using the least significant difference multiple comparison procedure.

Results and Discussion

Growth rate of all pigs was good over the duration of the 28 d study. Daily feed intake and feed-to-gain ratio were not affected ($P > .1$) by supplementation of the vitamin E sources (Table 2-2); however, a slight improvement in growth rate ($P < .09$) was obtained in pigs fed the D-α-tocopherol form compared to the negative control. Asghar et al. (1991) reported improved growth rates in growing-finishing pigs fed dietary levels of DL-α-tocopheryl acetate at 100 IU/kg of diet compared to pigs fed 10 IU/kg of diet. In contrast, Chung et al. (1992) found no difference in

Table 2-2. Performance of finishing pigs fed diets containing various vitamin E compounds

Item	Vitamin E source[a]				
	DL-α-TAC	D-α-TAC	DL-α-TOH	D-α-TOH	Neg. control
No. of pigs	8	8	8	8	8
ADG, kg	1.05[b]	1.0[b]	1.09[bc]	1.18[c]	1.02[b]
ADF, kg	3.87	3.5	3.66	3.79	3.4
F/G	3.78	3.61	3.37	3.28	3.55

[a]DL-α-TAC = DL-α-tocopheryl acetate; D-α-TAC = D-α-tocopheryl acetate; DL-α-TOH = DL-α-tocopherol; D-α-TOH = D-α-tocopherol.

[bc]Means within the same row with a different superscript differ significantly ($P < .09$).

growth performance due to vitamin E source (encapsulated D-α-tocopherol or DL-α-tocopheryl acetate) or level (16, 48 and 96 IU/kg) in trials with young, starting swine.

Alpha-tocopherol analysis of the diets containing the vitamin E forms are reported in Table 2-3. The vitamin E forms were included in the diet so that pigs would consume 62 IU of added vitamin E/kg of feed (72 IU/kg total). However, there was considerable variation in analyzed levels among the dietary treatments. Also, there was some variation in feed consumption among treatments. Therefore, data reported were adjusted based on analysis of diets and mean treatment group feed consumption (Table 2-3).

The indicator used to determine biopotency of the vitamin E compounds was the concentration of α-tocopherol in serum and selected tissues. Bratzler et al. (1950) found that plasma tocopherol concentration reflected level of tocopherol ingested in trials with young pigs fed different concentrations of tocopherol. They also observed increases of tocopherol in various tissues. Other researchers indicated that an animal's vitamin E status can be determined by measuring α-tocopherol concentration in serum and various tissue after oral administration: Baker et al. (1986) with humans, Hidiroglou and McDowell (1987) with sheep, and Jensen et al. (1990) and Asghar et al. (1991) with pigs. Numerous studies have shown that dietary vitamin E compounds are effective in elevating blood tocopherol

Table 2-3. Vitamin E (α-tocopherol) concentrations in feed

Treatment (vitamin E source)	Sampling day[a] 0	5	14	21
	----------IU/kg----------			
DL-α-tocopheryl acetate	94(72)	70(54)	64(49)	56(43)
D-α-tocopheryl acetate	113(72)	104(66)	74(47)	72(46)
DL-α-tocopherol	94(72)	24(17)	21(16)	13(10)
D-α-tocopherol	83(72)	22(19)	19(16)	15(13)
Neg. control	10	5	5	5

[a]Day samples were taken after start of trial. Samples were taken directly from feeder then frozen until analyzed. Numbers in parenthesis represent adjusted levels - adjusted to a constant IU/kg extrapolated from d 0 levels.

concentration, and also that blood tocopherol concentration increased with increasing dietary level of vitamin E (Hidiroglou et al., 1988; Jensen et al., 1988; Behrens and Madere, 1991; Asghar et al., 1991; Chung et al., 1992).

As expected, mean serum concentrations of tocopherol at d 0 (baseline) were similar across all treatments (Table 2-4). All vitamin E compounds fed in this experiment increased ($P < .01$) serum tocopherol concentration. The increase in serum tocopherol concentration was rapid. The increase started on d 1, grew further by d 2 ($P < .01$), and plateaued by d 7. Horwitt et al. (1984) noted in a study with humans that serum α-tocopherol concentrations were increased at 8 to 24 hr after ingestion of various vitamin E forms. Howard et al. (1990) with pigs weaned at 28 d, depleted of vitamin E for the next 38 d, and then fed 30 IU of supplemental vitamin E in the form of D-α-tocopheryl acetate or DL-α-tocopheryl acetate per kg of diet noted a rapid increase in blood α-tocopherol. Jensen et al. (1990), in a study with pigs, 49 d old, also observed a rapid increase in serum tocopherol concentrations after feeding supplemental DL-α-tocopheryl acetate. In both of the above swine studies, the first blood samples were taken 7 d after the start of the feeding trial.

Serum tocopherol concentrations of pigs fed both acetate forms were maintained beyond d 7; however, levels dropped steadily in pigs fed the alcohol forms and were

Table 2-4. Adjusted serum vitamin E (tocopherol) concentrations in finishing swine fed diets supplemented with different vitamin E compounds

Day	Vitamin E source[a]				
	DL-α-TAC	D-α-TAC	DL-α-TOH	D-α-TOH	Neg. control
	μg/ml				
0	.8	.8	.8	.8	.9
1	.9[bc]	1.2[b]	1.1[b]	1.3[b]	.8[c]
2	1.3[c]	1.8[b]	1.4[c]	1.6[bc]	.8[d]
7	1.5[c]	2.2[b]	1.4[c]	1.4[c]	.6[d]
14	1.4[c]	1.8[b]	1.1[c]	1.3[c]	.4[d]
21	1.4[c]	1.8[b]	.6[d]	.9[d]	.3[e]
28	1.4[c]	1.7[b]	.5[d]	.5[d]	.4[d]

Note: Each mean is based on eight observations. Adjusted to constant intake of 72 IU/kg diet based on d 0 feed analyses (Table 2-3). Day 0 serum values were not adjusted.

[a]DL-α-TAC = DL-α-tocopheryl acetate; D-α-TAC = D-α-tocopheryl acetate; DL-α-TOH = DL-α-tocopherol; D-α-TOH = D-α-tocopherol.

[bcde]Means within the same row with a different superscript differ ($P < .01$).

lower ($P < .01$) on d 21 and 28 than the acetate forms. This drop was probably due to poor stability of the alcohol forms in the feed (Table 2-3). Degradation of vitamin E occurs through oxidation, and is accelerated by light, alkali, heat and trace minerals (Ullrey, 1981; Erdman et al., 1988; Dove and Ewan, 1991; Hidiroglou et al., 1992). In the absence of oxygen, tocopherols are relatively heat, light, and alkali stable (Ullrey, 1981). Stability of α-tocopherol is increased by acylation of the compound (Ullrey, 1981). Acetate forms of vitamin E were found to be quite stable in feed in our study. Acetate forms of vitamin E have also been noted to be stable compounds by other researchers (Harris and Ludwig, 1949a,b; Ullrey, 1981; Dove and Ewan, 1991; Chung et al., 1992). In general, serum tocopherol concentrations observed in the present study were similar to those of other studies in which pigs were fed diets containing similar levels of added vitamin E (Jensen et al., 1988; Asghar et al., 1991).

While all vitamin E forms evaluated rapidly increased serum tocopherol concentrations, there was some evidence of a slight difference in the rate of this increase. Average serum concentration in pigs fed the DL acetate form was not increased ($P > .05$) until d 2, whereas serum concentrations of pigs fed the other compounds were increased ($P < .05$) on d 1. Horwitt et al. (1984) found that D-tocopherol raised blood α-tocopherol concentrations faster than the D- or DL-

α-tocopherol acetate forms in research done with humans.

D-α-tocopheryl acetate resulted in higher serum tocopherol levels than the DL- acetate form. Relative to DL-α-tocopheryl acetate, the D- form of the same compound had an average biopotency of 146% (IU basis; Table 2-5) or 199% (weight basis; 146 X 1.36). Howard et al. (1990) determined a relative biopotency of 218% (weight basis) for D-α-tocopheryl acetate relative to the DL- form of the same compound in trials with growing pigs. Thus it would appear that the D- acetate form has a higher biopotency for swine than that determined from the traditional rat fetal-resorption bioassays. However, Ames (1979) presented evidence that the commonly accepted conversion value of 1.36 may be too low in trials evaluating the relative biopotency of several vitamin E compounds using the rat fetal-resorption assay. Both alcohol forms in our study exhibited similar biopotencies, and there was evidence during the early portion of the study that these forms were slightly more biopotent than DL-α-tocopherol acetate (Table 2-5).

Alpha-tocopherol in tissues in general followed a similar pattern to that observed with serum (Table 2-6). Overall, pigs fed any of the compounds had tissue tocopherol concentrations higher than the negative control. Other researchers have also found that adding vitamin E to the diet increased tissue concentrations (Bratzler et al., 1950; Jensen et al., 1990; Asghar et al., 1991).

Table 2-5. Relative biopotency of vitamin E compounds (%)

Compound	day 1	day 2	day 7	day 14	day 21	day 28	Avg.
D-α-TAC	154	146	151	151	141	132	146
DL-α-TOH	122	110	123	124	32	83	99
D-α-TOH	124	114	118	140	46	73	103

Note: Based on serum values. DL-α-tocopheryl acetate = 100; IU basis.

Table 2-6. Adjusted tissue α-tocopherol concentrations in finishing swine fed diets supplemented with different vitamin E compounds

	Vitamin E Source[a]				
Tissue	DL-α-TAC	D-α-TAC	DL-α-TOH	D-α-TOH	Neg. Control
	μg/g				
Liver	4.2[b]	4.9[b]	2.6[c]	2.7[c]	1.3[d]
Leaf fat	2.6	2.4	2.3	1.5	1.4
Back Fat	2.4[bc]	2.8[b]	1.9[cd]	.9[e]	1.3[de]
Rhomboideus	2.6[b]	2.9[b]	1.6[cd]	1.9[c]	1.4[d]
Semimembranosus	1.0[cd]	2.5[b]	1.3[cd]	1.4[c]	.9[d]

Note: Each mean is based on four observations. Adjusted to constant intake of 72 IU/kg diet based on d 0 analyses (Table 2-3). Negative control values not adjusted.

[a]DL-α-TAC = DL-α-tocopheryl acetate; D-α-TAC = D-α-tocopheryl acetate; DL-α-TOH = DL-α-tocopherol; D-α-TOH = D-α-tocopherol.

[bcdde]Means within the same row with a different superscript differ significantly ($P < .02$).

Generally, acetate vitamin E forms resulted in greater tissue tocopherol concentrations than alcohol forms, and the difference was significant ($P < .02$) in the liver and rhomboideus. The greater tissue levels reflected the greater stability of the acetate forms in the feed over the duration of the study. Liver tocopherol averaged 4.6 μg/g for the acetate forms and 2.6 μg/g for the alcohol forms. Between the two acetate forms, D-α-tocopheryl acetate resulted in the same tissue tocopherol concentrations as the DL- form, with exception of the semimembranosus in which the D- form resulted in higher concentrations ($P < .02$). Tissue tocopherol concentrations were similar for the two alcohol forms.

Liver had the highest tocopherol concentration of all tissues evaluated. The liver could be an indicator of dietary vitamin E status or reflect immediate status. Jensen et al. (1990) indicated that serum and liver α-tocopherol concentrations reflected the short term vitamin E status of the pig and that muscle and fat tissue concentrations reflected the pig's long-term vitamin E status.

The concentration of tocopherol in all tissues other than the liver was similar ($P > .10$) in pigs fed the two acetate forms, with the exception of semimembranosus as noted above. Jensen et al. (1988), in trials with growing pigs, also noted that the liver had the highest

concentration of tocopherol, followed by adipose tissue and skeletal muscle. Also, Asghar et al. (1991), feeding growing pigs DL-α-tocopheryl acetate at 100 IU/kg of diet, observed higher tocopherol concentrations in the liver, followed by the heart, lung and kidney.

In conclusion, all vitamin E compounds evaluated almost immediately (by d 1) began to increase serum α-tocopherol concentrations upon ingestion. Tissue α-tocopherol concentrations were reflective of serum concentrations. Vitamin E acetate forms fed to finishing pigs resulted in higher serum and tissue tocopherol concentrations than the alcohol forms due to their greater stability in mixed feed. Among the two acetate forms evaluated, the D- form had a greater biopotency for swine than that determined by traditional assays.

Summary

Relative biopotencies of four chemical forms of vitamin E supplemented in diets of finishing swine for 28 d were evaluated. Forty crossbred pigs (80 kg), individually penned, were divided equally among five treatments. Treatments consisted of corn-soybean meal based diets supplemented with DL-α-tocopherol, DL-α-tocopheryl acetate, D-α-tocopherol or D-α-tocopheryl acetate. A treatment without vitamin E supplementation (negative control) served as the fifth treatment. Each compound was supplemented at

62 IU/kg of diet. Blood samples were collected on d 0, 1, 2, 7, 14, 21, and 28. On d 29, half of the pigs were slaughtered to obtain tissue samples. Feed samples, taken from feeders, were collected on d 0, 5, 14, and 21. All vitamin E forms fed increased ($P < .05$) serum α-tocopherol concentration by d 2 and the concentration peaked by d 7. Serum tocopherol concentrations in pigs fed either acetate form remained elevated beyond d 7; serum concentrations steadily declined and were lower ($P < .01$) on d 21 and 28 in pigs fed either alcohol form in comparison to acetate-fed pigs. The decrease was probably a reflection of reduced stability of the alcohol forms in the feed; the acetate forms were found to be stable in the feed over the 28 d study. Dietary supplementation of D-α-tocopheryl acetate resulted in the highest serum tocopherol concentrations throughout the study, compared to concentrations obtained for pigs fed the other compounds. A similar trend was observed in tissue (liver, back fat, leaf fat, semimembranosus, rhomboideus) tocopherol concentrations as with serum concentrations, with the liver having the highest concentration. The biopotency of D-α-tocopheryl acetate for swine appears to be greater than predicted from traditional bioassays with rats.

CHAPTER 3
THE EFFECT OF EXCESSIVE DIETARY VITAMIN A ON PERFORMANCE AND VITAMIN E STATUS IN SWINE FED DIETS VARYING IN DIETARY VITAMIN E

Introduction

Both vitamins A and E are fat soluble vitamins. There is evidence that high dietary vitamin A may interfere with both vitamin E absorption and blood α-tocopherol concentration. High dietary vitamin A reduced absorption of α-tocopherol in trials with chicks (Sklan and Donoghue, 1982). Abawi and Sullivan (1989) noted decreased plasma vitamin E concentration when chicks received high (100,000 IU/kg) levels of dietary vitamin A. Blakely et al. (1991) also reported that high dietary vitamin A (100 times requirement) plus high levels of beta carotene (480 mg/kg of diet) reduced plasma vitamin E concentration by 77% in rats.

Limited research is available on the effect of dietary vitamin A on vitamin E status in pigs. Our study was done to evaluate the effect of excessive dietary vitamin A on performance and on serum and tissue concentrations of α-tocopherol of growing-finishing pigs fed diets supplemented with varying levels of vitamin E.

Experimental Procedures

Eighty-four crossbred pigs with an average initial weight of 26 kg were divided by sex, weight and litter origin into pens of two pigs each (1 barrow, 1 gilt). Each pen was assigned to one of six dietary treatments within each of seven replications. The treatments for the 2 x 3 trial consisted of a basal corn-soybean meal diet supplemented with DL-α-tocopheryl acetate (Hoffmann-La Roche Inc., Nutley, NJ) at levels of 0, 15, or 150 IU/kg, and retinyl acetate (Hoffmann-La Roche Inc., Nutley, NJ) at levels of 2,000 or 20,000 IU/kg of diet. Pigs were fed a grower diet (Table 3-1), formulated to meet NRC (1988) requirements (except for vitamins A and E), until they reached an average body weight of 57 kg and continued on a finisher diet (Table 3-1) with the same treatment until they reached an averaged body weight of 107 kg. The vitamin premix used did not contain vitamins A and E. Pigs were given feed and water ad libitum. Pigs were housed in an open-sided building in pens with solid concrete floors. Pigs were weighed at the start of the feeding phase and biweekly thereafter. Average daily weight gains, feed to gain ratios, and average daily feed intakes were determined. Prior to the start of the study the pigs were fed a nursery diet that contained 2750 IU of added vitamin A and 22 IU added vitamin E/kg of feed. The trial was carried out

Table 3-1. Composition of diets (%)

Ingredient	Grower	Finisher
Ground corn	75.00	82.05
Soybean meal (48%)	22.00	15.00
Dynafos	1.70	1.70
Ground limestone	.80	.80
Salt	.25	.25
Trace mineral premix[a]	.10	.10
Vitamin premix	.10[b]	.05[c]
Se premix[d]	.05	.05

[a]Provided 200 ppm zinc, 100 ppm iron, 55 ppm manganese, 11 ppm copper, and 1.5 ppm iodine.

[b]Provided 4.4 mg riboflavin, 22 mg niacin, 18 mg pantothenic acid, 300 mg choline chloride, 22 ug vitamin B_{12}, 3 mg vitamin K, and 880 IU vitamin D_3 per kg of diet.

[c]Provided 2.2 mg riboflavin, 11 mg niacin, 9 mg pantothenic acid, 150 mg choline chloride, 11 ug vitamin B_{12}, 1.5 mg vitamin K, and 440 IU vitamin D_3 per kg of diet.

[d]Provided .1 ppm selenium.

during late spring and early summer (March through June). Pigs were managed following acceptable care and management practices. Protocol for animal care had been approved by the University Animal Use Committee.

Blood samples were collected by jugular venipuncture from each pig at the start (d 0) and on d 3, 7, 21, 35, 63, and 77 of the feeding period thereafter. Blood samples were shielded from direct sunlight. Blood samples were centrifuged after collection, serum harvested, frozen and stored at -20°C until analyzed. During storage, blood samples were covered with foil to avoid exposure to light.

Upon termination of the feeding phase, one pig (barrow) per pen was slaughtered, following accepted slaughter procedures, at the University of Florida meats laboratory and tissue samples collected. Tissue samples included liver, leg (semimembranosus) and neck (rhomboideus) muscle, back fat (10th rib area) and leaf fat. Tissue samples were frozen following collection and stored at -20°C until analyzed.

Vitamin E (α-tocopherol) was extracted from serum samples using the procedure described by McMurray and Blanchflower (1979a) with modifications described by Njeru et al. (1992). Extraction of vitamin E from tissue and feed samples was done using the procedure of Chung et al. (1992). This procedure was similar to that of McMurray and Blanchflower (1979b) and Hatam and Kayden (1979) with

modifications (Njeru et al., 1992). Alpha-tocopherol was determined by injecting 20 ul of the reconstituted sample (serum, or tissue) into an HPLC (Anderson et al., In Press). Alpha-tocopherol concentration of samples was calculated from the known concentration of standards. Spiked samples of α-tocopherol were found to have a mean recovery rate of 97 ± 3%.

Vitamin A was extracted from serum and tissues as described by Chew et al. (1991) and Mooney (1992). Extraction procedures were performed under dark conditions with either yellow filtered or subdued light. Vitamin A was assayed and determined by the method of Mooney (1992). The only modifications were that HPLC prepared samples were eluted using an 80:20 (vol/vol) mixture of iso-octane: tetrahydrofuran with 1% acetic acid and retention time was approximately 10.5 min. All trans retinol (Sigma Chemical Co., St. Louis, MO) standards were prepared and used to determine concentration of samples. Several liver samples were spiked to determine the recovery rate and validate the extraction procedure. Recovery rate of retinol was found to be 100 ± 4.7%.

Data collection included serum and tissue concentrations of vitamin A and E and performance data (body weight gain, feed-to-gain and feed intake). Tissue data were log transformed prior to analysis to improve homogeneity of variance. A univariate repeated measures

ANOVA was performed on serum data. Data were analyzed using the GLM procedure of SAS (1988). Orthogonal polynomial contrasts were performed to compare treatment means.

Results and Discussion

Growth performance data of the pigs in this study are summarized in Table 3-2. Pigs grew well on all dietary treatments (NRC, 1988). An increase in average daily gain of pigs approached significance ($P = .15$) in linear fashion as dietary vitamin E increased; feed to gain was not affected ($P > .1$). Dietary vitamin A levels had no effect on pig performance ($P > .1$). Hoppe et al. (1992) fed pigs 54 IU of supplemental vitamin E in combination with 5,000, 10,000, 20,000 or 40,000 IU of retinol/kg of diet and also observed no differences in pig performance. Other researchers have noted similar results in the chick (Sklan and Donoghue, 1982; Abawi and Sullivan, 1989) and rat (Blakely et al., 1991).

Serum α-tocopherol concentrations are reported in Tables 3-3 and 3-4. Serum tocopherol concentrations were affected by dietary α-tocopherol on all sampling days except d 0 (Table 3-4). When no supplemental vitamin E was added to the diet, there was a steady decline in serum tocopherol concentration from d 0 to d 77 (Table 3-4). When the diet was supplemented with 15 IU of α-tocopheryl acetate per kg of diet, serum tocopherol concentrations declined ($P < .05$)

Table 3-2. Performance of growing-finishing swine fed diets with different dietary levels of vitamin E and Vitamin A

Added Vit. E, IU/kg	Added vitamin A, IU/kg 2,000	20,000	Mean	SE
	Avg. daily gain, kg[a]			
0	.91	.91	.91	
15	.92	.96	.94	
150	.96	.92	.95	
Mean	.93	.94		.01
SE			.02	
	Avg. daily feed, kg[b]			
0	3.02	3.23	3.13	
15	3.10	3.11	3.11	
150	3.17	3.08	3.12	
Mean	3.09	3.14		.05
SE			.09	
	Avg. feed/gain[c]			
0	3.33	3.56	3.44	
15	3.39	3.25	3.32	
150	3.29	3.32	3.30	
Mean	3.34	3.38		.07
SE			.08	

Note: Seven pens per treatment with 2 pigs per pen.

[a]P>F:E = .19, A = .75, E*A = .22, E linear = .15

[b]P>F:E = .97, A = .52, E*A = .26, E linear = .94

[c]P>F:E = .41, A = .67, E*A = .26, E linear = .36

Table 3-3. Mean α-tocopherol concentrations in blood serum due to dietary additions of vitamins E and A

	Vit. E and A, IU/kg[a]						
Days	0/2[b]	0/20[b]	15/2	15/20	150/2	150/20	SE
	μg/ml						
0	1.14	1.17	1.18	1.24	1.03	1.25	.11
3	.60	.66	.75	.75	2.75	2.04	.09
7	.55	.59	.92	1.02	3.18	2.92	.11
21	.37	.53	.75	.84	3.15	2.99	.10
35	.39	.45	.87	.78	3.18	2.99	.09
63	.31	.32	.99	.78	3.29	3.24	.08
77	.33	.31	.98	.70	2.84	3.02	.11

Note: Each mean is based on 14 observations.

[a]Vitamin E linear effect $P < .01$ all days except d 0. E x A $P < .01$ d 3 and 21 only.

[b]2 = 2,000; 20 = 20,000 IU of retinyl acetate.

Table 3-4. Main means of serum α-tocopherol due to dietary additions of vitamins E and A

	Vit. E[a], IU/kg				Vit. A[b], IU/kg		
Days	0	15	150	SE	2,000	20,000	SE
	μg/ml						
0	1.15	1.21	1.14	.07	1.11	1.22	.06
3	.63	.75	2.40	.06	1.37	1.15	.05
7	.57	.97	3.05	.08	1.55	1.51	.06
21	.45	.79	2.92	.07	1.42	1.35	.06
35	.41	.83	3.08	.06	1.48	1.40	.05
63	.31	.88	3.26	.05	1.53	1.45	.04
77	.31	.84	2.93	.08	1.38	1.34	.06

Note: Each mean is based on 28 or 42 observations.

[a]Vitamin E linear effect $P < .01$ all days except d 0; E quadratic $P < .02$, d 63 and 77 only.

[b]Vitamin A effect $P < .01$, d 3 only.

from their initial concentration by d 3 and then stabilized throughout the remainder of the trial (Table 3-4). Serum tocopherol concentration increased ($P < .01$) with the highest vitamin E supplementation level by d 3 and continued to increase ($P < .01$) to d 7 after which serum concentration was maintained throughout the study (Table 3-4). Serum tocopherol was highest ($P < .01$) at the highest level of vitamin E supplementation on all days except d 0. Overall, as dietary levels of supplemental vitamin E increased, serum concentration of tocopherol also increased (linear; $P < .01$). Other studies have shown dietary vitamin E compounds are effective in increasing blood tocopherol and that blood concentrations increased with increasing dietary vitamin E (Jensen et al., 1988; Asghar et al., 1991 and Anderson et al., In Press).

Serum tocopherol concentrations due to supplementing the diet with vitamin A at 2,000 or 20,000 IU/kg of diet are summarized in Table 3-4. Pigs fed the low level of vitamin A tended to have higher concentrations of serum tocopherol than pigs fed the high level of vitamin A on all blood sampling days, but was significant only on d 3 ($P < .01$). Among the three dietary levels of vitamin E, the highest dietary vitamin E tended to be decreased by the high dietary vitamin A to the greatest extent, but was significantly lower ($P < .01$) on d 3 and d 21 only (Table 3-3). Although significant differences were noted above, the magnitude of

these changes was very small. Therefore vitamin A level of 20,000 IU/kg had only a minimal effect on blood tocopherol in swine. Weaver et al. (1989), in a trial with young starting pigs, observed that high dietary levels of vitamin A (9,900 and 16,500 IU/kg) fed with 33 IU of added vitamin E/kg tended to lower plasma tocopherol concentrations slightly. In trials with chicks and rats, however, a decrease in blood tocopherol was noted upon feeding diets with excessive vitamin A. In these trials, very high vitamin A concentrations (> 100,000 IU/kg of diet) were fed (Sklan and Donoghue, 1982; Blakely et al., 1991). This negative effect is thought to be attributed to competition for absorption sites in the small intestine or enhanced oxidation of tocopherol prior to tocopherol reaching the small intestine (Sklan and Donoghue, 1982).

Although some differences were observed on individual sampling days, supplemental vitamin A had no effect ($P > .10$) on serum retinol (Table 3-5). Differences that were noted were very small and within normal values for serum retinol usually found in the pig (Kaneko, 1980). Blood retinol was also not affected by dietary supplementation of various levels of vitamin A in studies done by other researchers (Abawi and Sullivan, 1989; Blakely et al., 1991; Hoppe et al., 1992).

With two exceptions, there was no effect ($P > .10$) on serum retinol due to supplementation of vitamin E at any of

Table 3-5. Main means of serum retinol due to dietary additions of vitamins E and A

	Vit. E[a], IU/kg				Vit. A[b], IU/kg		
Days	0	15	150	SE	2,000	20,000	SE
	----------μg/ml----------						
0	.34	.34	.39	.02	.31	.41	.02
3	.31	.33	.35	.01	.30	.35	.01
7	.34	.32	.35	.01	.33	.34	.01
21	.34	.36	.35	.01	.35	.35	.01
35	.37	.34	.32	.01	.35	.34	.01
63	.39	.35	.37	.01	.38	.36	.01
77	.47	.49	.46	.02	.52	.44	.02

Note: Each mean is based on 28 or 42 observations.

[a]Vitamin E linear effect $P < .05$ on d 3 and 35 only.

[b]Vitamin A effect $P < .01$ on d 3 and 77 only.

the dietary levels evaluated in this study. Increasing dietary vitamin E (Table 3-5) resulted in higher ($P < .02$) retinol serum concentration on d 3 in pigs fed the highest supplemental vitamin E, but also resulted in the lowest ($P < .02$) serum retinol concentration on d 35. Although both were significant, the differences were small. Weaver et al. (1989) in trials with growing pigs also noted that plasma vitamin A was not affected by dietary level of vitamin E.

Tissue α-tocopherol increased (linear; $P < .001$) as dietary vitamin E increased (Table 3-6). As dietary vitamin E increased from 15 to 150 IU per kg of diet, tissue tocopherol increased by at least a factor of 2 or more in all tissues evaluated. This finding is in agreement with others who have observed similar responses in pigs (Jensen et al., 1988; Asghar et al., 1991). Among the tissues sampled, the highest concentration of α-tocopherol due to treatment was found in the adipose tissue followed by liver and muscle tissue, respectively (Table 3-6).

High supplementation of vitamin A had no effect ($P > .1$) on tocopherol concentration in any of the tissues studied (Table 3-6). The retinol concentration in the liver was greatly enhanced ($P < .001$) when pigs were fed the high level of vitamin A (Table 3-7). Retinol concentration in other tissues (back fat, leaf fat, rhomboideus and semimembranosus) was not detected and are not reported. Dietary vitamin E had no effect ($P > .10$) on liver retinol

Table 3-6. Main means of tissue α-tocopherol concentrations due to dietary additions of vitamins E and A

Tissue	Vit. E[a], IU/kg				Vit. A, IU/kg		
	0	15	150	SE	2,000	20,000	SE
	μg/g						
Semimembranosus	.4[a]	1.4[b]	4.0[c]	.14	1.9	2.0	.11
Rhomboideus	.3[a]	2.0[b]	6.5[c]	.30	2.9	2.9	.24
Back fat	1.1[a]	2.7[b]	11.0[c]	.35	5.2	4.5	.28
Leaf fat	.9[a]	3.1[b]	10.0[c]	.26	4.8	4.5	.21
Liver	1.0[a]	2.6[a]	7.2[b]	.65	3.8	3.5	.53

Note: Each mean is based on 14 or 21 observations.

[a]Vitamin E linear effect $P < .001$ for all tissues.

Table 3-7. Main means of tissue retinol concentrations due to dietary additions of vitamins E and A

	Vit. E, IU/kg				Vit. A[a], IU/kg		
Tissue	0	15	150	SE	2,000	20,000	SE
	μg/g						
Liver	503	470	496	44	103	876	36

Note: Each mean is based on 14 or 21 observations.

[a]Vitamin A effect $P < .001$.

concentration (Table 3-7). Hoppe et al. (1992) found that liver retinol was linearly related to dietary retinol in a trial involving young, growing pigs as was also observed in the present study. They also found that 10,000 IU of dietary retinol did not affect tissue α-tocopherol other than the heart in which α-tocopherol was slightly depressed. However, Pudelkiewicz et al. (1964) feeding vitamin A depleted chicks 0, 1,453, 14,535, 1,453,488 and 14,534,883 IU of vitamin A acetate/kg of diet observed a marked decline in tocopherol concentration in liver tissue at the highest dietary vitamin A levels.

In conclusion, there was no consistent evidence that excessive dietary vitamin A (20,000 IU/kg of diet) affected growth performance or serum or tissue α-tocopherol in growing-finishing pigs fed diets supplemented with varying levels of vitamin E. Likewise serum retinol was not affected by dietary vitamin E.

Summary

A 2 X 3 factorial experiment was conducted to evaluate excessive dietary vitamin A on vitamin E status and performance of growing-finishing pigs fed diets supplemented with varying levels of vitamin E. Treatments consisted of corn-soybean meal based diets supplemented with DL-α-tocopheryl acetate to provide 0, 15 or 150 IU added vitamin E/kg and with retinyl acetate to provide 2,000 or 20,000 IU

vitamin A/kg of diet. The trial involved 84 crossbred pigs (26 kg) divided by sex, weight, and genetic background into pens of two pigs each. Treatment was assigned at random to pens within each of seven replications. Pigs were fed grower diets (.75% lysine) until they reached 57 kg average weight and were then switched to finisher diets (.60%) until 107 kg. Serum was collected on day 0, 3, 7, 21, 35, 63, and 77 of the feeding period. Tissue samples (liver, muscle, back fat and leaf fat) were collected from one pig (barrow) in each pen at the end of the feeding phase. Overall average daily gain and feed-to-gain were .93 kg and 3.35 respectively, without treatment differences ($P > .1$). Excessive dietary vitamin A had no effect ($P > .1$) on serum retinol concentrations except d 3 in which there was a small ($P < .01$) increase. Serum tocopherol was increased ($P < .01$; linear) by d 3 with dietary vitamin E supplementation and was maintained ($P < .01$) throughout the feeding period. High dietary vitamin A resulted in a small but significant ($P < .01$) decrease in serum tocopherol on d 3; serum tocopherol concentrations were not affected on other days. Tissue tocopherol was increased ($P < .001$; linear) as dietary vitamin E increased from 15 to 150 IU/kg. Liver retinol increased ($P < .001$) by a factor of eight. No consistent evidence was found that high dietary vitamin A interfered with performance or with serum or tissue tocopherol in growing-finishing swine.

CHAPTER 4
EFFECT OF INJECTED VITAMIN A AND DIETARY SUPPLEMENTATION OF VITAMIN E ON REPRODUCTIVE PERFORMANCE AND TOCOPHEROL STATUS IN GESTATING GILTS

Introduction

Supplemental vitamin A and/or ß-carotene given via injection just before and/or shortly after breeding appears to enhance reproductive performance of gilts and sows (Brief and Chew, 1985; Coffey and Britt, 1993). There is evidence that high dietary vitamin A may interfere with both vitamin E absorption and blood α-tocopherol concentrations. High dietary vitamin A reduced absorption of α-tocopherol in trials with chicks (Sklan and Donoghue, 1982). Abawi and Sullivan (1989) noted a decrease in plasma vitamin E concentrations when depleted chicks were administered high (100,000 IU/kg) levels of dietary vitamin A. Blakely et al. (1991) also reported that high levels of vitamin A (100 times requirement) plus high levels of ß-carotene (480 mg/kg of diet) reduced vitamin E plasma concentration by 77% in rats.

This study was conducted to evaluate the effect of injecting vitamin A just before, during and shortly after breeding, and dietary supplementation of vitamin E on reproductive performance and on blood and tissue

concentrations of α-tocopherol during early gestation of gilts.

Experimental Procedures

The trial was a 2 x 2 factorial design and involved 32 (7 to 8 month old) crossbred gilts. The gilts were divided into pens of 8 gilts each. Gilts used in this study were from a previous trial that involved the feeding of diets supplemented with either 2,000 (L) or 20,000 (H) IU vitamin A/kg of diet. This was taken into consideration in the allotment of gilts to treatment (4L and 4H per pen). Each pen was randomly assigned to one of four treatments. Treatments consisted of a basal corn soybean meal diet (Table 4-1) supplemented with DL-α-tocopheryl acetate (Hoffmann-La Roche Inc. Nutley, NJ) at levels of either 25 or 500 IU/kg of diet. Gilts were fed experimental diets beginning 7 d prior to breeding through d 25 of gestation. Half of the gilts were given three injections (i.m. in the neck) of 350,000 IU each of vitamin A (vitamin A palmitate Hoffmann-La Roche Inc. Nutley, NJ); the other half were injected with vehicle only. The gilts were injected at 7 d prebreeding (d -7), at breeding (d 0) and 7 d postbreeding (d 7). Gilts were fed 1.9 kg of feed/hd once daily and given free access to water. Gilts were housed in an open-sided building with solid concrete floors. Gilts were checked twice daily for estrus during the trial and doubled

Table 4-1. Composition of diet fed to gestating gilts

Ingredient	%
Ground corn	84.66
Soybean meal (48%)	12.40
Dynafos	1.54
Ground limestone	.65
Salt	.50
Trace mineral[a]	.10
Vitamin premix[b]	.10
Se premix[c]	.05

[a]Provided 200 ppm zinc, 100 ppm iron, 55 ppm manganese, 11 ppm copper, 1.5 ppm iodine, and 1 ppm cobalt.

[b]Provided 4.4 mg riboflavin, 22 mg niacin, 18 mg pantothenic acid, 300 mg choline chloride, 22 ug vitamin B_{12}, 3 mg vitamin K, 880 IU vitamin D_3, and 4000 IU vitamin A per kg of diet.

[c]Provided .1 ppm selenium.

mated on their second or third observed estrus to duroc x hampshire x yorkshire boars.

Blood samples were collected by jugular vein puncture from each gilt on d -7, 0, 7 and 24 of gestation to monitor the serum α-tocopherol (vitamin E) and retinol (vitamin A) concentrations. Blood samples were covered with foil to prevent exposure to direct sunlight, taken to the laboratory, centrifuged and the serum harvested. Serum was stored at -20°C until analyzed for α-tocopherol and retinol concentration. During storage, serum samples were covered with foil to prevent exposure to light.

Gilts were slaughtered, following accepted slaughter procedures, on d 25 of gestation at the University of Florida meats laboratory. Reproductive tracts were immediately removed and refrigerated for later counting of corpora lutea (CL) and recovery of embryos. Tissue samples were also collected which consisted of endometrium, embryo, ovary, uterus, liver, leaf fat, back fat and muscle (semimembranosus and rhomboideus). Tissue samples were stored at -20°C until analyzed for α-tocopherol and retinol concentrations.

The trial was carried out during the summer and early fall (July through October). Pigs were managed following acceptable care and management practices throughout the study. Protocol for animal care had been approved by the University Animal Use Committee. Three pigs from different

treatment groups were eliminated from the study due to death, lameness and failure to conceive.

Alpha-tocopherol was extracted from serum and tissues using procedures as described earlier (Anderson et al., In Press). Alpha-tocopherol concentration was determined by injecting 10 ul of the extracted sample (serum and tissue) into the HPLC system. Retinol was extracted, assayed and concentration determined from the serum and tissues by the method as described previously (Mooney, 1992).

Experimental data included serum and tissue concentrations of α-tocopherol and retinol, and reproductive performance. Tissue data was log transformed prior to analysis to improve homogeneity of variance. A univariate repeated measures ANOVA was performed on serum data (SAS, 1988). Data were analyzed as a 2 X 2 factorial design with the factors being dietary vitamin E level and whether or not gilts were injected with vitamin A.

Results and Discussion

Levels of vitamin E used were chosen to reflect NRC (1988) requirement and to give a very high level. The injected level for vitamin A was chosen because it was thought to be the upper limit that would elicit a response and not be toxic to the gilts (also some evidence of this dosage was being used).

Reproductive performance data are summarized in Table

Table 4-2. Mean reproductive response criteria of gestating gilts given dietary additions of vitamin E and injected with vitamin A

	Vit. E, IU/kg and Vit. A inj.[a]				
Item	25/No	25/Yes	500/No	500/Yes	SE
No. of CL	14	13	15	15	.76
No. of Embryo[b]	12	13	12	15	1.13
Embryo wt., g	9	9	7	8	.98
Ovary wt., g	13	13	14	15	.90
Uterus wt., kg	3	2	2	2	.22

Note: Each mean is based on 7 or 8 observations.

[a]Three injections of 350,000 IU each.

[b]Effect of vitamin E (P = .14), effect of vitamin A (P = .13).

4-2. Although none of the differences noted with reproductive data were significant (P > .1), due to the small number of gilts per treatment and the inherent nature of swine reproductive data, nevertheless, some positive trends were observed due to treatment. Gilts receiving the high vitamin E and high vitamin A treatment had larger (P = .16) litters than gilts given other treatments. Embryonic survival was 86% in gilts given the low dietary vitamin E with no injected vitamin A and 80% in gilts given the high dietary vitamin E with no injected A. Embryonic survival was 100% in gilts injected with vitamin A and fed either the low or high vitamin E diets. Brief and Chew (1985) reported larger litter size and higher embryonic survival in gilts receiving weekly injections of vitamin A (12,300 IU) and ß-carotene (33 mg) compared to gilts fed vitamin A and ß-carotene at the same levels. However, these gilts in the study of Brief and Chew (1985) were depleted of vitamin A and ß-carotene for 5 weeks before the start of the study. Coffey and Britt (1993) observed on average .5 pig increase in the number of pigs born alive and higher embryonic survival in sows given i.m. injections of vitamin A palmitate (50,000 IU) compared to sows given vehicle only (corn oil) on day of weaning, mating and 7 d postbreeding. These sows were also supplemented with 11,000 IU of vitamin A acetate per kg of diet.

Serum concentrations of α-tocopherol in gilts fed diets

supplemented with two levels of vitamin E with and without injected vitamin A are reported in Tables 4-3 and 4-4. Initial α-tocopherol serum concentrations in gilts were similar (d -7). When dietary levels were increased from 25 to 500 IU of DL-α-tocopheryl acetate per kg of diet, serum α-tocopherol concentrations increased ($P < .01$) by d 0 and were maintained throughout the duration of the study (Table 4-4). This finding is in agreement with other studies that have shown that dietary vitamin E compounds are effective in increasing serum tocopherol, and that serum concentration increased with increasing dietary vitamin E (Jensen et al., 1988; Asghar et al., 1991; Mahan, 1991 and Anderson et al., In Press).

Serum α-tocopherol was not affected ($P > .1$) by injecting gilts with vitamin A (retinyl palmitate) except on d 7 (Table 4-4). On d 7, the gilts fed the low vitamin E diet had similar ($P > .1$) serum tocopherol concentrations regardless of vitamin A treatment, whereas gilts fed the high vitamin E diet had higher ($P < .08$) serum tocopherol concentrations when injected with vitamin A than gilts not injected with vitamin A (Table 4-3).

Serum concentrations of retinol due to treatment are shown in Tables 4-5 and 4-6. In general, there was no consistent effect on serum retinol due to dietary vitamin E supplementation level or injection of vitamin A. However, a difference in serum retinol ($P < .08$) was observed on d 0 in

Table 4-3. Mean serum α-tocopherol concentrations in gestating gilts given dietary additions of vitamin E and injected with vitamin A

	Vit. E, IU/kg and vit. A inj.[a,b,c]				
Sampling day	25/No	25/Yes	500/No	500/Yes	SE
	----------μg/ml----------				
-7	.8	.9	1.1	.6	.17
0	1.2	1.2	3.6	3.6	.18
7	1.1	1.1	3.4	4.0	.15
24	1.3	1.3	3.8	3.6	.15

Note: Each mean is based on 7 or 8 observations.

[a]Three injections of 350,000 IU each.

[b]Effect of vitamin E ($P < .01$), d 0, 7, 24.

[c]E x A effect ($P < .08$), d 7.

Table 4-4. Main mean serum α-tocopherol concentrations in gestating gilts given dietary additions of vitamin E and injected with vitamin A

Sampling day	Vit. E, IU/kg[a]		Vit. A inj.[b,c]		
	25	500	No	Yes	SE
	μg/ml				
-7	.8	.9	.9	.8	.12
0	1.2	3.6	2.4	2.4	.13
7	1.1	3.7	2.3	2.6	.11
24	1.3	3.7	2.5	2.5	.11

Note: Each mean is based on 14, 15 or 16 observations.

[a]Effect of vitamin E ($P < .01$), d 0, 7 and 24.

[b]Three injections of 350,000 IU each.

[c]Effect of vitamin A ($P < .06$), d 7 only.

Table 4-5. Mean serum retinol concentrations in gestating gilts given dietary additions of vitamin E and injected with vitamin A

Sampling day	Vit. E, IU/kg[a] and vit. A inj.[b,c]				SE
	25/No	25/Yes	500/No	500/Yes	
	----------μg/ml----------				
-7	.51	.55	.52	.51	.03
0	.44	.49	.51	.59	.03
7	.40	.43	.47	.42	.03
24	.46	.47	.48	.45	.03

Note: Each mean is based on 7 or 8 observations.

[a]Effect of vitamin E ($P < .03$), d 0 only.

[b]Three injections of 350,000 IU each.

[c]Effect of vitamin A ($P < .08$), d 0 only.

Table 4-6. Main mean serum retinol concentrations in gestating gilts given dietary additions of vitamin E and injected with vitamin A

Sampling day	Vit. E, IU/kg[a]		Vit. A inj.[b,c]		SE
	25	500	No	Yes	
	μg/ml				
-7	.53	.52	.52	.53	.02
0	.46	.55	.47	.54	.02
7	.41	.44	.43	.42	.02
24	.46	.47	.47	.46	.01

Note: Each mean is based on 14, 15 or 16 observations.

[a]Vitamin E effect ($P < .03$) d 0 only.

[b]Three injections of 350,000 IU each.

[c]Vitamin A effect ($P < .08$) d 0 only.

that serum retinol concentration was highest in gilts fed the high vitamin E and injected with vitamin A, and lowest in gilts fed low vitamin E without injected A. The difference noted was very small and within normal values for serum retinol concentrations usually found in the pig. Our findings agree with Weaver et al. (1989) in that plasma vitamin A concentration was not affected by dietary level of vitamin E. Serum retinol concentrations in the chick and rat have also been reported not to be affected by dietary supplementation of vitamin E in studies done by Abawi and Sullivan, (1989) and Blakely et al. (1991), respectively. Mooney (1992) injected gilts with vitamin A palmitate (ranging from 53,200 to 106,400 IU given once weekly), or ß-carotene (106.4 to 425.6 mg) and observed no difference in plasma concentrations of either retinol or ß-carotene. In contrast, Brief and Chew (1985) noted increased plasma vitamin A concentration upon injecting vitamin A, however, gilts used in their research were depleted of vitamin A prior to the study and they also received injected ß-carotene. Serum retinol may have been elevated with injection of vitamin A early in our study but may have been missed since blood samples were taken 7 d after injection.

Tissue α-tocopherol concentration in gestating gilts increased ($P < .01$) as dietary supplementation of vitamin E increased (Table 4-7) in all tissues except adipose. This finding is in agreement with others who have observed

Table 4-7. Mean tissue α-tocopherol concentrations of gestating gilts given dietary additions of vitamin E and injected with vitamin A

Tissue[a]	Vit. E, IU/kg and vit. A inj.[b]				
	25/No	25/Yes	500/No	500/Yes	SE
	----------μg/g[c]----------				
Liver	4	4	24	23	1.6
Back fat	6	8	9	9	1.5
Leaf fat	9	10	12	12	2.2
Semimembranosus	2	3	4	4	.3
Rhomboideus	3	3	7	7	.6
Endometrium[d]	2	1	4	5	.3
Embryo	.4	.4	.8	.7	.05
Oviduct	1	1	4	3	.3
Uterus	1	1	4	4	.2
Ovary	20	19	104	90	5.6

Note: Each mean is based on 7 or 8 observations.

[a]Effect of vitamin E ($P < .01$) for all tissues except back fat and leaf fat.

[b]Three injections of 350,000 IU each.

[c]Wet tissue basis.

[d]Effect of vitamin A ($P < .08$); E x A ($P < .04$).

similar responses in the pig (Jensen et al., 1988; Asghar et al., 1991; Mahan, 1991). Average tocopherol concentration increased by a factor of 2 in embryos upon high dietary supplementation indicating that tocopherol is transferred from the dam to the developing embryo. Vitamin A injections had no effect ($P > .1$) on tissue α-tocopherol concentrations except in the endometrium where there was a vitamin E x vitamin A interaction ($P < .04$). In the endometrium gilts fed low vitamin E and injected with vitamin A had slightly lowered tocopherol concentration, while gilts fed the high vitamin E and injected with vitamin A had increased tocopherol concentration over that of the non injected gilts (Table 4-7). Vitamin A injections appear to have no effect on the transfer of tocopherol into the developing embryos as tocopherol concentration in the embryos was not influenced by vitamin A injection ($P > .1$).

Injecting vitamin A had no effect ($P > .1$) on retinol concentration in any of the tissues studied including the liver. Retinol concentrations in tissues other than liver, however, were very small or nonexistent. Average concentration in the liver was 386 μg/g. Mooney (1992) found no differences in concentration of retinol in uterine flushings in gilts that were injected with varying levels of vitamin A; liver retinol was not determined.

Among the tissues sampled, the highest average concentration of α-tocopherol upon supplementation of high

level of vitamin E was found in the ovary, followed by liver, adipose, rhomboideus and endometrium, respectively (Table 4-8). Three other tissues followed (semimembranosus, oviduct, uterus) that had similar average concentrations and embryo tissue had the lowest α-tocopherol concentration (units per wet tissue basis).

In conclusion, there was no consistent evidence found in this study that injecting a relatively large amount of vitamin A (3 injections of 350,000 IU) just before, during and shortly after breeding, significantly improved reproductive performance, or interfered with serum or tissue concentrations of α-tocopherol in gestating gilts fed diets supplemented with 25 or 500 IU of vitamin E/kg of diet. However, tocopherol concentration was increased further in the endometrial tissue when vitamin A was given along with high dietary vitamin E. No evidence was found that injections of vitamin A interfered with the transfer of α-tocopherol to the developing embryo. Likewise, serum retinol concentrations were not affected by treatment.

Summary

A 2 × 2 factorial experiment was conducted to determine the effects of injecting vitamin A and feeding vitamin E on reproductive performance and on blood serum and tissue concentrations of tocopherol during early gestation of gilts. Thirty-two crossbred gilts were fed corn soybean-

Table 4-8. Main mean tissue α-tocopherol concentrations in gestating gilts given dietary additions of vitamin E and injected with vitamin A

Tissues[a]	Vit. E, IU/kg		Vit. A inj.[b]		
	25	500	No	Yes	SE
	μg/g[c]				
Liver	4	24	14	14	1.1
Back fat	7	9	7	9	1.0
Leaf fat	9	12	10	11	1.5
Semimembranosus	3	4	3	3	.2
Rhomboideus	3	7	5	5	.4
Endometrium[d]	1.4	4.6	2.8	3.3	.18
Embryo	.4	.8	.6	.6	.03
Oviduct	1	4	3	2	.2
Uterus	1	4	3	3	.1
Ovary	19	97	61	55	3.8

Note: Each mean is based on 14, 15 or 16 observations.

[a]Effect of vitamin E ($P < .01$) for all tissues except back fat and leaf fat.

[b]Three injections of 350,000 IU each.

[c]Wet tissue basis.

[d]Effect of vitamin A ($P < .08$).

meal based diets supplemented with DL-α-tocopheryl acetate to provide either 25 or 500 IU of added vitamin E/kg of diet. Gilts were fed daily 1.9 kg/h beginning d -7 prebreeding through d 25 of gestation. Half of the gilts were injected (i.m.) with 350,000 IU of retinol palmitate at d -7 prebreeding, breeding (d 0) and d 7 postbreeding; the other half were injected with vehicle only. All gilts were double mated during their second or third estrus. Blood samples were collected on d −7, 0, 7 and 24 of gestation. Gilts were slaughtered on d 25 of gestation following accepted slaughter procedures. Twenty-nine gilts conceived. The number of corpora lutea and embryos was not affected ($P > .1$) by treatment. Serum tocopherol concentrations increased with 500 IU of vitamin E by d 0 and were stable through d 24 of gestation ($P < .01$). Vitamin A injections had no effect ($P > .1$) on serum tocopherol concentrations except on d 7 when a small increase ($P < .06$) was noted. High dietary vitamin E increased tocopherol concentration ($P < .01$) in all tissues examined except fat. A vitamin E × vitamin A interaction ($P < .04$) was noted in endometrium tissue. Low dietary vitamin E and injections of vitamin A slightly lowered tocopherol concentration, while high vitamin E and vitamin A injections increased tocopherol concentration in the endometrium. Increasing dietary vitamin E increased serum and tissue tocopherol concentrations. Vitamin A injections had little or no

effect on these concentrations during early gestation of gilts.

CHAPTER 5
GENERAL CONCLUSIONS

Three experiments were conducted, one to determine the bioavailability of four forms of vitamin E compounds, and two to assess the effect of high levels of vitamin A on the vitamin E status of growing finishing pigs or gestating gilts.

In experiment 1 the biopotency of four forms of vitamin E were determined. Generally, the acetate forms resulted in greater serum and tissue concentrations of vitamin E (α-tocopherol) than the alcohol forms, due to the greater stability of the acetate forms that was noted in mixed feed. Serum tocopherol increased rather rapidly when the four compounds were fed. Dietary supplementation of D-α-tocopheryl acetate resulted in the highest serum tocopherol throughout the study, compared to concentrations obtained for pigs fed the other compounds indicating a greater biopotency (IU/mg) for swine than determined by the traditional rat bioassay. A similar trend was observed with tissue (liver, back fat, leaf fat, and muscle) tocopherol concentrations as with serum concentrations, with the liver having the highest concentration. In general, all forms would probably be suitable dietary supplemental sources if

the stability of the alcohols were improved. Encapsulating the alcohol forms to protect them from destruction would increase their suitability for use in mixed feed.

Experiment 2 was conducted to evaluate the effect of feeding excessive vitamin A on growth performance, and on blood and tissue α-tocopherol (vitamin E) levels of growing-finishing pigs. High dietary vitamin A (20,000 IU/kg of diet) was found not to affect or have little affect on pig performance, or on blood or tissue concentrations of α-tocopherol. A threefold increase ($P < .01$) in serum tocopherol occurred on all sampling days when dietary supplementation increased from 15 to 150 IU/kg. Tissue tocopherol also increased ($P < .001$) as dietary vitamin E increased from 15 to 150 IU/kg. Tissue tocopherol concentration increased ($P < .001$) by a factor of at least two in all tissues evaluated. Liver retinol increased ($P < .001$) eightfold with a tenfold increase in dietary vitamin A. Even in the liver when vitamin A (retinol) concentration was high, α-tocopherol concentration was not affected. Thus, the form of vitamin A within the liver is not in a form which can lead to oxidative destruction of α-tocopherol or the concentrations encountered may not have been high enough to affect α-tocopherol.

Experiment 3 evaluated the effect of giving a high level of vitamin A via intramuscular injections on reproductive performance, and on serum and tissue α-

tocopherol concentrations during early gestation of gilts. High levels of vitamin A (350,000 IU per week) did not affect reproductive performance, or serum or tissue concentrations of α-tocopherol or retinol in this study. As observed in the previous studies, increasing dietary levels of vitamin E increased blood serum and tissue tocopherol concentrations including reproductive tissues. Alpha-tocopherol concentration also increased in the embryo when dietary vitamin E was increased. The increased concentration of α-tocopherol indicates a transfer of tocopherol from the dam to the developing embryo. Fluctuations of retinol concentrations in serum may have been missed due to weekly sampling of blood. Blood sampling on d 2, 3, or on a more frequent routine after vitamin A injections would provide more answers.

Further research would be desirable to determine vitamin E bioavailability other than by oral administration. Fecal sample tocopherol analysis may provide more information from which better conclusions might be drawn concerning digestion and absorption. Studies using larger numbers of gilts and/or sows is recommended to determine if vitamin A injection alone or in combination with high vitamin E, either orally or by injection, would enhance reproductive performance.

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BIOGRAPHICAL SKETCH

Lee E. Anderson, Sr., was born December 1944 in Pineland, South Carolina. He is married to Erma L. Anderson and the father of five children. He graduated from Middleton Senior High School in 1962. He graduated with an Associate of Arts degree in 1964 from Gibbs Junior College. He received a Bachelor of Science in Agriculture in 1969 from Florida A and M University. He received the Master of Science in reproductive physiology from the University of Florida in 1972. Since receiving the M.S. he has been employed at Alcorn State University, Fort Valley State University and Florida A and M University, in extension, research and instructional programs. He is presently a candidate for the Ph.D.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Robert O. Myer, Chairman
Associate Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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